TAPROBANICA, ISSN 1800-427X. April, 2012. Vol. 04, No. 01: pp. 37-41.

GASTROINTESTINAL PARASITES OF CAPTIVE PRIMATES IN THE NATIONAL ZOOLOGICAL GARDENS OF SRI LANKA

Sectional Editor: Colin Chapman *Submitted*: 05 August 2011, *Accepted*: 15 April 2012

Umanga C. Gunasekera[1,3], Susiji Wickramasinghe[1], Ganga Wijesinghe[2] and R. P. V. J. Rajapakse[1]

[1] Department of Pathobiology, Faculty of Veterinary Medicine & Animal Science, University of Peradeniya, Sri Lanka
[2] The National Zoological Gardens of Sri Lanka, Dehiwala, Sri Lanka
E-mail: [3] umavet@yahoo.com

Abstract
Fifteen species of primates from different geographic areas are living in captivity at the National Zoological Gardens of Sri Lanka. As a result of limited space in the Zoo and ever increasing visitors, there is a possibility to increase the incidence of human animal contact. Therefore, it is important to identify potential parasitic infections that can be transferred from humans to animals and *vise versa*. In the present study, the primates were investigated for the gastrointestinal parasites. Total of 85 fecal samples were collected from all the species and examined for the presence of helminthes and protozoa. *Balantidium* sp., *Entamoeba coli*, *Giardia* sp., *Blastocystis* sp. and coccidial oocytes including *Cryptosporidium* sp. oocysts were identified. Furthermore, Nematodes and Cestodes were also recorded.

***Key words*:** Helminthes, nematodes, cestodes, protozoa, parasitic infections, oocytes.

Introduction

Several kinds of gastrointestinal parasites were reported in both captive and non-captive primates in the world (Brack, 1987; Bruno *et al.*, 2007; Hendricks, 1977; Muriuki *et al*., 1998; Soulsby, 1982; Tachibana *et al.*, 2009). In Sri Lanka few studies have been carried out on identifying the gastrointestinal parasites in the primates and only handful of publications available on the captive animals in the zoo (e.g. Amarasinghe *et al.*, 2009) while there are few on non-captive fauna in the zoo premises (Karunarathna *et al.*, 2007; 2008). From Sri Lankan primates helminthes protozoa are reported in *Macaca sinica* (Dewit *et al.*, 1991; Ekanayake *et al.*, 2004) in the wild. The reported species were *Trichostrongylus* sp., *Strongyloid* sp., *Oesophagustomum* spp., *Cestoda* sp. and *Hymenolepsis* sp. The identified ciliates were *Giardia* sp., *Balantidium* sp. *Entamoeba histolytica*, *Entamoeba coli*, *Entamoeba hartmanni* and *Blastocystis* spp. and the coccidian parasite *Cryptosporidium* sp. The genus level identification was done using the PCR method. *Cryptosporidium* and other protozoan infections (*Entamoeba* sp., *Iodamoeba* sp., *Chilomastix* sp. and *Balantidium*

sp.) in wild *Trachypithecus vetulus* are also reported (Ekanayake *et al.*, 2004).

Materials and Methods

***Study Area*:** The National Zoological Gardens is approximately 10 hectares in extent. It is located in the wet zone of Sri Lanka (6º51' 21.48" - 6º51' 30.30" N and 79º 52' 20.08" - 79º52' 33.99" E) (Karunarathna *et al.*, 2008) at a mean elevation of 25 m above sea level. The nearest city is Dehiwala (2 km) and its proximity to the city of Colombo (11 km) makes it an easily accessible location for the potential visitors (Weinman, 1957). There are 15 species of primates in the national zoo and apart from that large numbers of wild animal species are housed in the premises. Approximately 100 species of mammals, 110 species of birds, 35 species of reptiles, butterflies and marine vertebrates constitute this collection. There are 16 sections in the national zoo.

Sample collection: Study period was 7 months commencing from August 2009 up to February 2010. Total of 85 fecal samples were collected throughout the study period. Fresh voided fecal samples were collected from the ground in the morning and they were transferred to the laboratory. The number of animals in each cage and the number of samples collected from each cage was recorded (Table 1). Sample collection was done once per every month during the study period. For the transportation, air tight samples were kept in styrofoam boxes with ice.

Table 1: Details of the samples collected and examined.

Common name	Scientific name	No. of animals in each cage	No. of samples collected & examined
	Section 03		
Siamang gibbon	*Symphalangus syndactylus*	1	3
Grey langur	*Semnopithecus entellus*	1	-
Toque monkey	*Macaca sinica*	8	4
	Section 08		
Japanese monkey	*Macaca fuscata*	3	2
Formosan monkey	*Macaca cyclopis*	1	3
Siamang gibbon	*Symphalangus syndactylus*	1	-
Silver leaf monkey	*Trachypithecus cristatus*	7	6
White handed gibbon	*Hylobates lar*	1	2
Squirrel monkey	*Saimiris ciureus*	1	5
Capuchin	*Cebus capucinus*	1	6
Sooty mangabey	*Cercocebus aterrimus*	1	6
	Section 11		
Orang-utan	*Pongo pygmaeus*	4	5
Toque monkey	*Macaca sinica*	4	5
Grey langur	*Semnopithecus entellus*	2	4
Japanese monkey	*Macaca fuscata*	2	5
	Sections 13 and 14		
Chimpanzees	*Pan troglodytes*	6	5
	Section 15		
Hamadryas baboon	*Papio hamadryas*	1	4
Toque monkey	*Macaca sinica*	5	3
Hooded Capuchin	*Cebus apella*	1	3
Black cheeked white nosed monkey	*Cercopithecus ascanius*	1	2
Patas monkey	*Erythrocebus patas*	1	2
Hamadryas baboon	*Papio hamadryas*	1	4
Grey langur	*Semnopithecus entellus*	4	5

***Identification*:** To determine the presence of parasites/eggs/cyst, following techniques were performed.

Direct fecal smear observation

Iodine stain: Lugo's iodine was used.

To isolate helminthes eggs: salt flotation technique

Detection of protozoan cysts: sugar flotation technique and/or Acetic acid–ether concentration technique

Identification of the Cryptosporidium oocysts: Ziehl-Neelsen staining technique.

Species identification: PCR (Gene Amp® PCR system 9700) was done using genomic DNA and *E. coli* specific primers (Forward-5'-GAATGTCAAAGCTAATACTTGACG-3' and Reverse-5'GATTTCTACAATTCTCTTGGCATA-3'). Promega Wizard® Genomic purification kit was used for the DNA extraction.PCR conditions were used as previously described by Tachibana *et al*. (2009). Amplified products were visualized in 1.5% agarose gel containing Ethidum bromide. DNA ladder (100 bp) was used as a marker to determine the length of the amplicons.

Culturing procedure of the protozoa: After morphological identification for further identification of protozoa, some of the fresh positive samples are directly cultured in the modified Tanabe-Chiba medium at 37ºC. Two sub cultures were done every twenty four hours later consequently according to Nilles-Bije & Rivera (2009)

Results and discussion

In the present study, we have identified several species of protozoa (*Cryptosporidum* sp., *Balantidium* sp., *Blastocyst* sp., *Entamoeba* sp., *Giardia* sp., and coccidian) in the chimpanzee, orang-utan, hamadryas baboon, Japanese macaque, siamang, toque monkey, grey langur, silvered leaf monkey, sooty Mangabey and Formosan monkey (Fig.1). Two protozoan cysts were not identified morphologically (morpho 1, morpho 2) due to the variations in shape, size and the internal structures. In PCR study, we successfully amplified 180 bp in length fragment using *E. coli* specific primers. Therefore, we confirmed that the species as a pathogenic *E. coli*. Furthermore, Nematode larvae (hook worm) and eggs (*Ascaris*, *Strongyle* and *Trichuris* types) were identified in some of the primate species (Table 2).

Table 2: The helminth eggs protozoan cysts detected in each primate species

Primate species	Hook worm	Trichuris eggs	Strongyle eggs	Nematod larvae	Ascaris eggs	Crypto	Cocci	Balanti	Blast cyst	Entam spp.	*Giardia* spp.	Morph 1	Morpho 2
Symphalangus syndactylus			+ve		+ve		+ve						
Semnopithecus entellus	+ve									+ve	+ve	+ve	
Macaca sinica	+ve	+ve		+ve	+ve			+ve	+ve				+ve
Macaca fuscata						+ve			+ve	+ve			
Macaca cyclopis					+ve								+ve
Presbytis cristata										+ve			
Saimiri sciureus													
Cebus capucinus			+ve										
Cercocebus atys						+ve							
Pongo pygmaeus	+ve					+ve		+ve					
Pan troglodyte				+ve			+ve	+ve					
Papio hamadryas		+ve				+ve	+ve	+ve					
Cebus apella													
Cercopithecus ascanius													
Erythrocebus patas													

+ (positive)

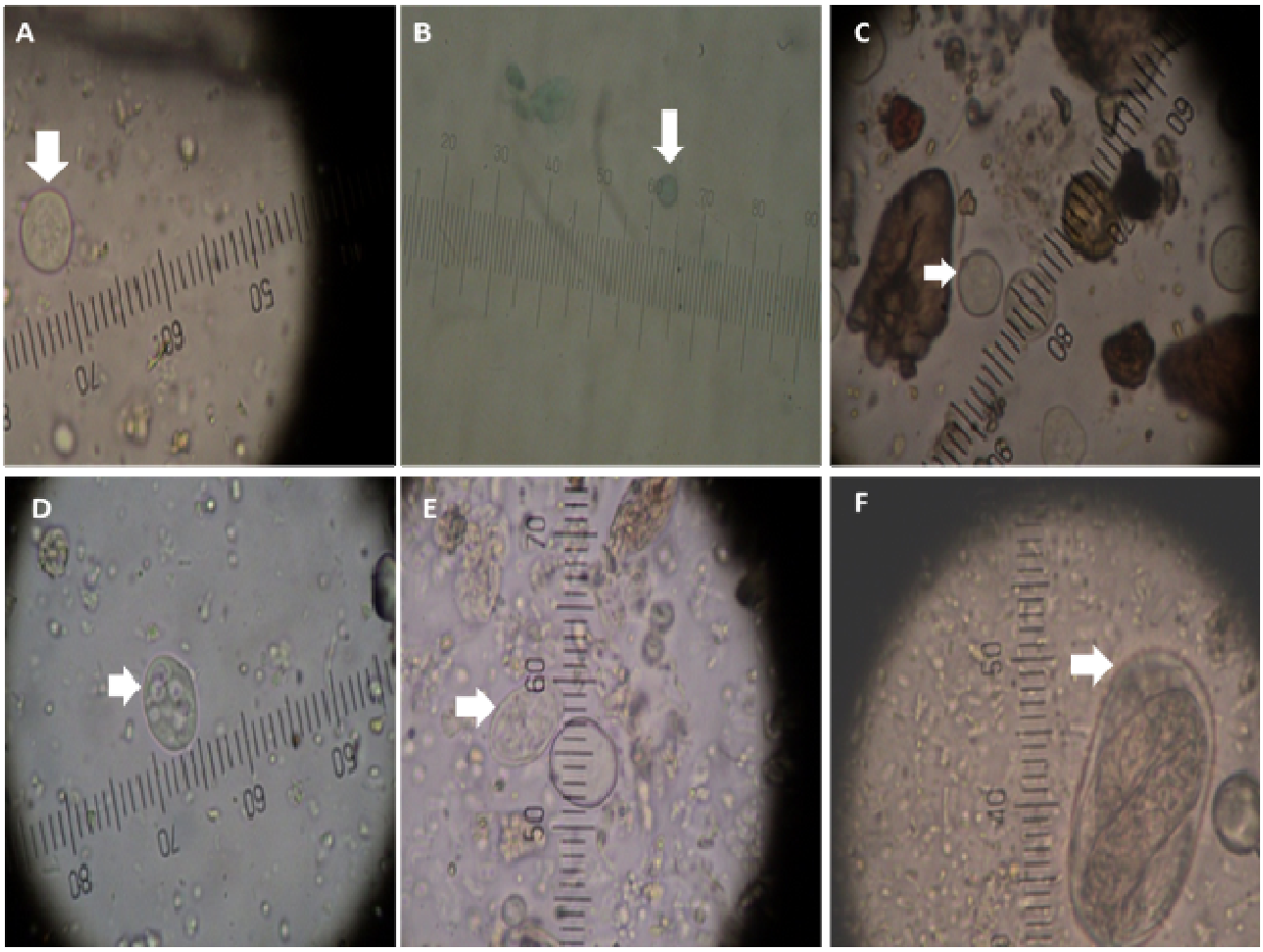

Figure 1: morphology of some of the protozoan cysts and nematode eggs identified (Magnification × 40) (**A**) Morpho 1 sp.; (**B**) *Blastocystis* cyst; (**C**) *Enatamoeba* cyst; (**D**) *Giardia* cyst; (**E**) *Nematode* egg; (**F**) *Cryptosporidium* cyst

The presences of gastrointestinal parasites have been reported in both captive and non-captive primates. Rarely, infected primates show clinical signs of particular parasitic diseases. More often, parasitic diseases are contracted when animals are immunosuppressed due to malnutrition, stress or as a result of heavy parasitic load.

Ziehl-Neelsen staining technique was used for the identification of *Cryptosporidium* oocysts. These oocysts were difficult to demonstrate during routine fecal examination because of the smaller size (size 2-6 µm). Therefore, acid-fast technique was used for the detection of oocysts. Originally the oocyst after staining should appear bright red. Due to the staining technical errors here it appears in slight pinkish colour. *Cryptosporidium* species is the most common protozoa found among the monkeys. It was reported that these monkeys are frequenting the areas and water that has been soiled by humans (Ekanayake *et al.*, 2004). Similarly, in our study, we found that *Cryptosporidium* sp. was presented in primates of Dehiwala Zoo. Furthermore, there were no new animals introduced to the already existing primate population during the study period. Therefore, there is a possibility that the primates have acquired it from contaminated food, human contact or environment. To confirm that it is the same parasite genus that is affecting both non-human primates and humans further detailed studies should be carried out at both morphological and molecular level. It is also possible that the primate is the preferred host and therefore the humans are not infected.

Gasser *et al.* (2004) screening of the workers for suspected zoonoses is also a must in the same manner. In this study most of the parasite identification has been done only up to the genus level. Further studies should be carried out to determine the zoonotic potential of the identified parasitic spp.

Coccidial oocysts were detected in the chimpanzee (*Pan troglodytes*). These primates had diarrhea and had been treated previously. It might be due to

cocccidial infection. Although, coccidial oocysts were recorded in several other species of primates during the study period however, they did not show any clinical signs. In North America, researchers have identified Coccidial oocysts in chimpanzee. Moreover, it has been confirmed as *Isospora* species based on morphology (Hendricks, 1977). *Cestodes* and *Trichuris* species were also found in some primates.

Balantidium species and *Ascaris* eggs were isolated in Toque monkeys. *Balantidium* species reported in our study is morphologically similar to *Balantidium coli*. In addition, we were able to identify *Giardia* sp., *Entamoeba coli* and *Blastocystis* sp. in most of the primates. However, they did not show any clinical signs of infection.

The culturing of the protozoa was done to perform a more accurate identification using the motile trophozoites. For culturing, both fresh and filtered positive samples were used. Filtered sample was used to reduce the inhibiting factors (Nilles-Bije & Rivera, 2009). However, it was not successful. This may be due to culturing of cysts instead of motile trophozoites or otherwise.

We suspect that primates acquired these parasitic infections mainly through contaminated foods or environment. Other concerns are stocking density of primates and the same keeper handling the different species of animals. Therefore, further studies are required to determine the source of parasitic infection.

Acknowledgements
Authors would like to thank K. B. A. T. Bandara for the technical assistance during the study.

Literature cited

Amarasinghe, A. A. T., M. S. Botejue, L. E. Harding, 2009. Social behaviours of captive *Trachypithecus cristatus* (mammalia: cercopithecidae) in the national zoological gardens of Sri Lanka. *Taprobanica*, 1 (1): 66-73.

Brack, M., 1987. *Agents transmissible from simians to man*. Springer-verlag, London: 454.

Bruno B. C., A. Belltto, X. M. Francois, 2007. Wild life, exotic pets and emerging zoonoses. *Emerging Infectious Disease*, 13 (1): 6-11.

Dewit I., P. J. Dittus, J. Vercruysse, A. Eileen, D. I. Gibson, 1991. Gastro-intestinal helminths in a natural population of *Macaca sinica* and *Presbytis* sp. at Polonnaruwa, Sri Lanka *Primates*, 32 (3): 391-395.

Ekanayake, K., A. Arulkanthan, N. U. Horadagoda, G. K. M. Sanjeevani, R. Kieft, S. Gunathilaka, P. J. Dittus, 2004. Prevalence of *Cryptosporidium* and other parasites enteric parasites among wild non–human primates in Polonnaruwa, Sri Lanka. *The American journal of tropical medicine and hygiene*, 74 (2); 322-329.

Gasser, R. B., J. M. de Gruijter and A. M. Polderman, 2009. The utility of molecular methods for elucidating primate-pathogen relationships the *Oesophagostomum bifurcum* example: 47-62. *In*: *Primate parasite ecology: the dynamics and study of host-parasite relationships*, M. A. Huffman and C. A. Chapman (eds.). Cambridge, UK, Cambridge University Press.

Hendricks, L. D., 1977. Host range characteristics of the primate coccidian, *Isospora arctopitheci* Rodhain, 1933 (Protozoa: Eimeriidae). *The Journal of Veterinary Parasitology*, 63: 32-33

Karunarathna, D. M. S. S., A. A. T. Amarasinghe, P. I. K. Pebotuwage and A. A. D. S. Udayakumara, 2007. A study of the non captive avifaunal diversity in the National Zoological Gardens, Dehiwala, Sri Lanka. *Siyoth*, 2 (2): 25-29.

Karunarathna, D. M. S. S., A. A. T. Amarasinghe and A. De Vos, 2008. Preliminary notes on the monitor lizards (Family: Varanidae) within the National Zoological Gardens (NZG) Dehiwala, Colombo District, Sri Lanka. *Biawak*, 2 (3): 109-118.

Muriuki S. M. K., Murugu R. K., Munene E., Karere G.M., Chai D.C., 1998. Some gastrointestinal parasites of zoonotic(public health) importance commonly observed in old world non-human primates in Kenya. *Acta Tropica* 71(1): 73-82.

Nilles-Bije, M. L. and L. W. Rivera, 2009. Ultra structural and molecular characterization of *Balantidium coli* isolated in the Philippines. *Parasitology Research*, 106: 387-394.

Soulsby, E. J. L., 1982. *Helminths, Arthropods and Protozoa of Domesticated Animals*. 6th edition, Bailliere Tindall, London: 56-58.

Tachibana, H., T. Yanagi, A. Akatsuka, S. Kobayashi, H. Kanbara, V. Tsutsumi, 2009. Isolation and characterization of potentially virulent species *Entamoeba nuttalli* from captive Japanese macaques. *Parasitology*, 136 (10): 1169-1177.

Weinman, A. N., 1957. *A zoological guide to the Zoological Gardens of Ceylon*. Government press of Ceylon: 73.